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PRINCIPAL INVESTIGATOR: Mina J. Bissell, Ph.D.

CONTRACTING ORGANIZATION: The University of California at Berkeley
Berkeley, CA 94720

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Mina J. Bissell, Ph.D.

E-mail: MJBissell@lbl.gov

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14. ABSTRACT

The goal of the present proposal is to provide post-doctoral training opportunities in breast cancer research that focus on the role of microenvironmental in mammary gland biology. Trainees will benefit from working in a dynamic interactive program under the guidance of the LBNL mentors to investigate the intersection of hormone action, growth factor activity, and extracellular matrix remodeling during mammary gland development and carcinogenesis. In addition, trainees will be exposed to a variety of other topics related to breast cancer, as well as research ranging from molecular medicine to genomics, by their participation in working groups, lectures and scientific meetings with other Berkeley Lab and Bay Area researchers.

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DOD TRAINING GRANT IN MAMMARY BIOLOGY – 2004/2005 ANNUAL REPORT

Introduction

The goal of this proposal was to provide post-doctoral training opportunities in breast cancer research that would focus on the role of microenvironment in mammary gland biology. Trainees benefited from working in a dynamic interactive program under the guidance of the LBNL mentors to investigate the intersection of hormone action, growth factor activity and extracellular matrix remodeling during mammary gland development and carcinogenesis using novel, physiological models. In addition, trainees were exposed to a variety of other topics related to breast cancer, as well as research ranging from molecular medicine to genomics, by their participation in working groups, lectures and scientific meetings with other Berkeley Lab and Bay Area researchers.

Postdoctoral trainees supported by this grant in 2005 included Aylin Rizki, Paraic Kenny and Mark LaBarge.

Postdoctoral Fellows/Research Accomplishments

- Aylin Rizki, Ph.D.

In this training period, Dr. Rizki was involved in two distinct but related projects:

One project tested the hypothesis that premalignant to malignant transition in breast cancer could be modeled using human breast cells in 3-D cultures. Ability to invade through a basement membrane is a defining step of premalignant to malignant conversion in breast cancer. To delineate molecular alterations necessary for this transition, we utilized loss of differentiation in three-dimensional laminin-rich extracellular matrix cultures as a screening tool and isolated cell lines that had lost the ability to form organized acini but remained non-invasive. These cells displayed high potential for acquiring invasiveness, low frequency of non-invasive tumor formation *in vivo*, and similarities to premalignant lesions *in vivo* as shown by their patterns of genomic aberration and gene expression. Inhibiting matrix metalloproteinases and integrins that were upregulated in the malignant derivatives abrogated their invasiveness. Using this novel model of premalignancy, we found that polo-like kinase 1 plays a role in acquisition of invasiveness through basement membrane in culture and *in vivo*, suggesting a new function for this mitotic kinase in extracellular matrix signaling.

The significance of the implications of this project is as follows: Transition from premalignant to malignant transition in human breast cancer progression is crucial since this is a prerequisite step to invasion and eventually metastasis, which is what kills the cancer patients. Determining which molecular alterations are necessary for the transition would provide useful clinical markers to distinguish benign lesions from those likely to progress, as well as providing functionally relevant molecular targets for treating early breast cancers. There have been no ex vivo spontaneous transition models to study molecular mechanisms of this transition to malignancy as it occurs in vivo. The three dimensional culture model developed here allowed us to determine which molecular changes between premalignant cells and their malignant derivatives are *causal* for transition to malignancy. The premalignant to malignant transition model provides both novel and effective tools for molecular dissection of how premalignant cells become breast cancer, as

exemplified by the identification of polo-like kinase 1 as a potential new target in treatment of premalignant breast disease.

The second project aimed to test the hypothesis that extracellular matrix signaling may play a role in maintaining genomic instability via effects on DNA repair. It has been observed by pathologists over many years that loss of cell-extracellular matrix interactions is one of the defining criteria for malignancy, as well as the parallel observation that genomic instability accompanies all breast cancers. One cause of genomic instability is altered double-strand break repair. Double-strand breaks can be repaired either by homologous recombination or non-homologous end-joining, both of which can lead to error-prone repair depending on genomic location, whether or not repeat sequences are involved, and the sub-pathways used in repair. Here we showed that laminin-rich extracellular matrix (lrECM) signals to downregulate the potentially mutagenic homologous recombinational repair of an endonuclease-induced double-strand break within a direct repeat substrate in both dividing and non-dividing non-tumorigenic breast epithelial cells. The downregulation was dependent on extracellular matrix signaling via the beta1 integrin receptor, since blocking this integrin stimulated HR and stimulating it downregulated homologous repair. We also showed that lrECM alters DNA damage response to ionizing radiation. Kinetics of phosphorylated histone H2AX foci formation after ionizing radiation was significantly altered by lrECM, in a beta1 integrin (an important subunit of ECM-receptors) -dependent manner. This difference was not due to a lower number of double-strand break formation after irradiation, since we found that lrECM has no effect on the amount of broken chromosomal DNA immediately after irradiation, as measured by pulsed-field gel electrophoresis. Long term clonogenic survival after treatment with ionizing radiation was enhanced by lrECM in non-dividing cells. These results implicate beta1 integrin-mediated ECM signaling as a novel pathway that regulates DNA damage response and repair.

The significance of this project lies in the fact that efficiency and fidelity of DNA repair contribute to the transition from normal to malignant breast, as well as being important in how well breast cancer patients respond to radiation therapy. Therefore, uncovering novel pathways that regulate DNA repair would further our knowledge of a fundamental process that often goes awry in tumorigenesis and may provide new targets for therapies.

Various aspects of this work were presented at four meetings (see reportable outcomes) and are currently in the form a revised manuscript submitted to Cancer Cell by Rizki A, Weaver VM, Chin K, Moonlee S-Y, Rozenberg G, Myers CA, Bascom JL, Mott JD, Jensen RA, Semeiks J, Grate LR, Mian IS, Petersen OW, Chen DJ, Chen F, Gray JW, Bissell MJ. Identification of functionally significant changes in transition from premalignant to malignant phenotype. Another paper is in preparation on the DNA repair data.

- Paraic Kenny, Ph.D.

During this last funding period, Dr. Kenny has concentrated on a model of human breast cancer progression, HMT3522, and the determination of the mechanisms by which the malignant cells of this series (T4-2) acquire self-sufficiency for EGF. Preliminary data developed with initial support from the Susan G. Komen Foundation showed that these cells upregulate two EGFR ligands, Amphiregulin and TGFα. In the current funding period, he has defined a critical requirement for the activity of a co-expressed protease (TACE/ADAM17) for the function of

these growth factors. Inhibition of this protease results in the reversion of the malignant phenotype of these cells in a 3D ECM culture assay. This is a phenocopy of EGFR inhibition and suggests a novel way of targeting this receptor tyrosine kinase pathway in tumors of the breast and of other epithelial tissues. He has extended this analysis to expression analysis of human breast cancer patient samples and demonstrated that expression of TGFα and TACE is predictive of poor prognosis in these patients (n=295).
Various aspects of this work were presented at several meetings (see reportable outcomes). A manuscript describing these findings was submitted to Cancer Cell and we are currently preparing a resubmission in response to reviewers' comments. **Kenny PA** and Bissell MJ (2005) Identification and targeting of a TACE-dependent autocrine loop which predicts poor prognosis in breast cancer.

- Mark LaBarge, Ph.D.

During this funding period Dr. LaBarge has combined his experience in adult stem cell biology during his Ph.D. program at Stanford with the Bissell lab's 3D culture models and cell signaling expertise to disect the molecular mechanisms which allow adult mammary epithelial and stem cells to integrate multiple instructional inputs from the surrounding microenvironment into single phenotypic outcomes. This project has taken two approaches: 1) We are investigating a putative form of cancer therapy in which malignant cells are forced to differentiate, termed reversion, and the signaling mechanisms that are involved in this process. 2) We are investigating how mammary stem cells interpret information in their microenvironment that directs them to differentiate into the two major mammary lineages, myo- and luminal-epithelial cells.

With regard to reversion and signal integration, Dr. LaBarge has chosen to focus on the EGFR/MAPK pathway, a prototypical proliferation pathway, and the Notch pathway, a prototypical differentiation pathway. Using the HMT3522 malignancy progression series in 3D culture, he has shown that Notch pathway antagonists can mediate tumor reversion; gamma secretase inhibitors, Numb overexpression, and dominant negative Numb-targeted E3 ligases, all mediate reversion of the T4-2 cell line. Conversely, Notch pathway agonists stimulate growth of the malignant cells in 3D culture and antagonize the well documented reverting effects of EGFR inhibitors, suggesting that these two pathways interact and require a certain balance of signaling input to achieve reversion. Indeed, as shown previously for EGFR and beta1 integrin, down regulation of either Notch or EGFR pathway activities lead to the reciprocal down regulation of the other pathway, as determined by western blot against acitve Notch1 and EGFR. Using shRNA expressed from a retrovirus, Dr. LaBarge has knocked out Numb and shown that it is required to mediate the cross modulation between Notch and EGFR pathways. These data are providing the initial evidence that the inter-relationship of the Notch and EGFR pathways is important in mammary acinus development and is a pathway worthy of more attention during the remainder of the granting period.

The work on the adult breast stem cell has just begun.

Training Activities

The trainees were exposed to a wide range of research approaches, tools, and methods that are encompassed in the mentor's laboratories. In addition to weekly **laboratory meetings** with the

preceptor (Dr. Bissell's meetings are two hours per week), a monthly **Cancer Biology department meeting** is held to bring together the investigators and the trainees to discuss research and literature relevant to the program. The department will hosts an annual a Postdoctoral Research Day which features poster presentations and a speaker chosen by postdoctoral fellows (http://www.lbl.gov/lifesciences/postdoc/index.htm contains the details of the LSD Postdoc Society). **Division seminars** are held weekly (see http://www.lbl.gov/lifesciences/resources/seminars.html for a roster of speakers for 2004).

Of particular relevance is the monthly **Mammary Gland Affinity Group**, which is a long standing tradition. LBNL mammary biology and breast cancer groups meet for informal research presentations. Additional participants from UC San Francisco Medial Center and UC Berkeley campus attend regularly. Approximately 30-40 participate. The format consists of two short talks by postdoctoral fellows.

The Life Sciences Division currently hosts approximately 50 research grants in breast cancer and mammary biology, totaling over $16 million in funds. Dr. Mina Bissell is the Principal Investigator of the Training Grant and past Director of the Life Sciences. The current Director, Dr. Joe Gray, maintains a joint appointment with the UCSF Cancer Center where he is the program leader of the Breast Oncology Program. This program contains the NCI-funded Bay Area Breast Cancer Specialized Program Of Research Excellence (SPORE). http://cc.ucsf.edu/breast_spore/index.html. Drs. Gray and Bissell also now have an NCI U54 grant. The Breast Oncology Program has a weekly seminar series which we now video conference to LBNL. These seminars provide the postdoctoral fellows with a good mixture of basic research and clinical research. It also provides a good opportunity for the postdoctoral fellows to hear and understand the concerns of breast cancer advocates.

Reportable Outcomes

Publications: Two manuscripts are in revision for Cancer Cell and many posters and talks were presented at scientific meetings. See Appendices.

Appendix 1

Additional Publications

Bascom JL and ***Kenny PA** (2005) Meeting Report - Keystone Symposium: The Role of Microenvironment in Tumor Induction and Progression, Banff, Canada, Feb 5th – Feb 10th 2005. Breast Cancer Research 7: 113-118

Kenny PA and Bissell MJ (2005) Identification and targeting of a TACE-dependent autocrine loop which predicts poor prognosis in breast cancer (Submitted)

Bissell MJ, **Kenny PA**, Radisky DC (2005) Microenvironmental regulators of tissue structure and tumor progression – The role of matrix metalloproteinases in the loss of tissue polarity and induction of genomic instability. Cold Spring Harbor Symposia in Quantitative Biology (in press, 2005)

Appendix 2

Conference Abstracts

#1

American Association for Cancer Research (AACR)
Radiation Biology and Cancer: From Molecular Responses to the Clinic
February 18-22, 2004, Dana Point, CA

Double-strand Break Repair in Human Breast Epithelial Cells is Regulated by Extracellular Matrix Signaling

Aylin Rizki[1], Bjorn Rydberg[1], Paul D. Kaufman[1,2], David J. Chen[1], Fanqing Chen[1], Maria Jasin[3], Mina J. Bissell[1].

[1]Department of Molecular and Cell Biology, Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720
[2] Division of Genetics and Development, University of California, Berkeley, California
[3]Division of Cell Biology, Sloan-Kettering Institute, New York, New York

Genomic instability and altered cell - extracellular matrix (ECM) interactions are two features that accompany almost all breast cancers. One of the causes of genomic instability is altered double-strand break (DSB) repair. DSBs can be repaired either by homologous recombination or non-homologous end-joining (NHEJ). However, apart from cell cycle effects, it is not clear what regulates the choice of DSB repair pathway employed by a damaged cell. We propose ECM-initiated signaling is a likely candidate for this. We base this hypothesis on the observation that ECM alters the organization of the nucleus. Minimally, homology-directed events are likely to be affected when the proximity of homologous sequences is altered by ECM-mediated nuclear re-organization. We employed three approaches to investigate the effects of ECM signaling on DSB repair. First, we incorporated a direct repeat GFP substrate which allowed detection of homologous repair of an I-SceI induced break by formation of functional GFP sequences. We found that addition of ECM causes a decrease in the frequency of homologous recombinational repair of the I-SceI break by 2 and 3 fold on average in dividing and non-dividing cells, respectively. Our second approach has been to ask if ECM alters break induction, repair, and survival in response to X-ray irradiation. We irradiated cells that were growth arrested either in the presence or absence of ECM and then we allowed them to repair. We found that cells that were irradiated in the presence of ECM had two-fold lower clonogenic survival than their counterparts treated in the absence of ECM. In preliminary experiments using PFGE, we determined the number of breaks induced and remaining two hours after X-irradiation and found that repair in growth arrested cells in the presence of ECM appeared to be more efficient compared to cells grown in the absence of ECM, suggesting that fast-repair (NHEJ) is stimulated by ECM. We are currently investigating if slow repair and repair of X-ray damage in dividing cells is altered by ECM. Our third approach has been to determine global gene expression changes induced by ECM and to ask which DNA repair-related genes have altered expression. cDNA microarray results show that ECM decreases mRNA levels of XRCC3, RAD52 and CHAF1, and increases the level of MRE11. We are investigating the functional significance of these changes in mRNA level to the regulation of DSB repair by ECM.

#2

AACR International Conference on Tumor Progression and Therapeutic Resistance
November 8-9, 2004, Philadelphia, PA

Extracellular Matrix Signaling in Breast Cancer Progression and in DNA Double-Strand Break Repair

Aylin Rizki[1], Valerie M. Weaver[2], Bjorn Rydberg[1], Koei Chin[3], Sun-Young Moonlee[1], Gabriela I. Rozenberg[2], Connie A. Myers[1], Jamie L. Bascom[1], Joni D. Mott[1], Jeremy R. Semeiks[1], Leslie R. Grate[1], I. Saira Mian[1], Roy A. Jensen[4], Ole W. Petersen [5], David J. Chen[1], Fanqing Chen[1], Maria Jasin[6] Joe W. Gray[1,3], Mina J. Bissell[1]

[1]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720
[2]Department of Pathology and Institute for Medicine and Engineering, University of Pennsylvania, PA 19104
[3] Department of Laboratory Medicine and Comprehensive Cancer Center, University of California, San Francisco, CA 94143
[4]Kansas Masonic Cancer Research Institute, Kansas City, KS 66160
[5]The Panum Institute, DK-2200 Copenhagen N, Denmark
[6] Division of Cell Biology, Sloan-Kettering Institute, New York, NY 10021

The ability to invade a basement membrane is a defining step of premalignant to malignant conversion in breast cancer. To delineate molecular alterations necessary for this transition, we utilized loss of differentiation in three-dimensional laminin-rich basement membrane (3DlrBM) cultures as a screening tool and isolated cell lines that have lost the ability to form organized acini but remain non-invasive. These cells displayed high potential for acquiring invasiveness, low frequency of non-invasive tumor formation *in vivo*, and similarities to premalignant lesions *in vivo* as shown by their patterns of genomic aberration and gene expression. Inhibiting matrix metalloproteinases and integrins that were upregulated in the malignant derivatives abrogated their invasiveness, implicating aberrant extracellular matrix signaling as a crucial step in the transition to malignancy. In addition to alterations in extracellular matrix signaling, genomic instability is a hallmark of most breast cancers. One cause of genomic instability is altered doublestrand break (DSB) repair. DSBs can be repaired either by homologous recombination or non-homologous end-joining (NHEJ). Here we showed that lrBM signals to downregulate HR of an I-SceI induced DSB in non-tumorigenic breast epithelial cells. Blocking β1 integrin stimulated HR and stimulating β 1 integrin downregulated HR. Kinetics of overall induction or repair of breaks induced by 40Gy IR was not significantly affected by lrBM as measured by PFGE, however, kinetics of γ-H2AX foci formation after 3 or 6Gy IR is significantly altered by lrBM. Clonogenic survival after 3, 6, or 10 Gy IR was either enhanced or inhibited by lrBM, depending on the growth and polarity of cells. These results implicate extracellular matrix signaling as a modifier of cellular response to radiation therapy in breast cancer via effects on DNA repair.

#3

California Breast Cancer Research Program -CBCRP
From Research to Action: Seeking Solutions, September 9-11, 2005, Sacramento, CA

Extracellular Matrix Signaling in Breast Cancer Progression and in DNA Double-Strand Break Repair

Aylin Rizki[1], Valerie M. Weaver[2], Bjorn Rydberg[1], Koei Chin[3], Sun-Young Moonlee[1], Gabriela I. Rozenberg[2], Connie A. Myers[1], Jamie L. Bascom[1], Joni D. Mott[1], Jeremy R. Semeiks[1], Leslie R. Grate[1], I. Saira Mian[1], Roy A. Jensen[4], Ole W. Petersen [5], David J. Chen[1], Fanqing Chen[1], Maria Jasin[6] Joe W. Gray[1,3], Mina J. Bissell[1]

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#4

FASEB Summer Conferences
FASEB Genetic Recombination and Genome Rearrangements
July 23-28, 2005, Snowmass Village, Colorado

Extracellular Matrix Signaling via Beta1 Integrin Regulates DNA Double-Strand Break Repair

Aylin Rizki[1], Bjorn Rydberg[1], Maria Jasin[2], Mina Bissell[1]

[1]Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, CA 94720.
[2]Memorial Sloan-Kettering Cancer Research Institute, New York, NY 10021.

Double-strand breaks (DSBs) can be repaired either by homologous recombination or non-homologous end-joining (NHEJ), both of which can lead to error prone repair depending on genomic location, whether or not repeat sequences are involved, and the sub-pathways used in repair. Here we showed that laminin-rich extracellular matrix (lrECM) signals to downregulate the potentially mutagenic HR of an endonuclease-induced DSB within a direct repeat substrate in both dividing and nondividing non-tumorigenic breast epithelial cells. The downregulation was dependent on ECM signaling via the beta1 integrin receptor, since blocking this integrin stimulated HR and stimulating it downregulated HR. We also showed that lrECM alters DNA damage response to ionizing radiation. Kinetics of phosphorylated H2AX foci formation after ionizing radiation was significantly altered by lrECM, in a beta1 integrin dependent manner. This difference was not due to a lower number of DSB formation after irradiation since we found that lrECM has no effect on the amount of broken chromosomal DNA immediately after irradiation, as measured by pulsed-field gel electrophoresis. Long term clonogenic survival after treatment with ionizing radiation was enhanced by lrECM in non-dividing cells. These results implicate beta1 integrin mediated ECM signaling as a novel pathway that regulates DNA damage response and repair.

Appendix 3

Meeting Abstracts

#1

Keystone Symposium: The role of microenvironment in tumor induction and progression
Banff, Canada, Feb 5-10, 2005

Coordinate upregulation of Amphiregulin, TGFβ and TACE mediates growth factor self-sufficiency in a human breast cancer progression model

Paraic A. Kenny and Mina J. Bissell

Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley CA 94720

Self sufficiency in growth factor receptor signaling is a fundamental hallmark of tumorigenesis. We have compared non-malignant cells ("S1"), and their malignant derivatives ("T4-2") of the HMT3522 breast cancer progression series to further explore this phenomenon. S1 cells form phenotypically normal, growth-arrested polarized structures of similar size and morphology to human breast acini when cultured in a 3D laminin-rich basement membrane microenvironment. Unlike S1 cells, T4-2 cells grow independently of exogenous EGF, forming highly proliferative, disorganized, apolar colonies in this 3D assay and are malignant in nude mice. Interruption of this signaling cascade in T4-2 cells by inhibition of EGFR, MAPK or PI3K results in reversion of the malignant phenotype: these cells arrest growth and form polarized acinus-like structures typical of non-malignant breast epithelial cells. We report that T4-2 cells acquire growth factor autonomy by transcriptional upregulation of two EGFR ligands, Amphiregulin and TGF□and that co-induction of an ADAM metalloproteinase not expressed in S1 cells, TACE/ADAM17, is necessary for AR and TGFβ function. Reversion of maligancy of T4-2 cells in culture is accompanied by downregulation of AR transcripts. Inhibition of TACE in T4-2 cells blocked growth factor mobilization and resulted in reversion in the 3D assay, essentially phenocopying EGFR inhibition. We further demonstrate that addition of TACE is both necessary and sufficient for signaling by endogenously overexpressed growth factors in non-malignant S1 cells.
The identification of this autocrine loop establishes the mechanism by which T4-2 cells acquired growth factor self-sufficiency and suggests that metalloproteinase inhibition may have therapeutic efficacy in those tumors which co-overexpress growth factors and receptors, a subset with a currently poor prognosis. These investigations were supported by a Susan G. Komen Breast Cancer Foundation Postdoctoral Fellowship (#2000-223) to PAK and by grants from the OBER office of the DOE, the NCI, and an Innovator Award and training grants from the DOD to MJB.

#2

Cold Spring Harbor Symposium in Quantitative Biology LXX
Cold Spring Harbor, NY, June 1-6, 2005

Targeting TACE-dependent growth factor shedding in breast cancer

Paraic A. Kenny and Mina J. Bissell
Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley CA 94720

The ability to proliferate independently of signals from other cell types is a fundamental characteristic of the tumor cell. Acquisition of this phenotype is likely necessary in all cancers, but the preferred mechanism by which it is achieved is somewhat tissue specific. While common in other epithelial tumors, mutations in components of the EGFR pathway such as Ras and Raf are rare in breast tumors. Here, we analyze a model of human breast cancer progression in which this transition has occurred without mutation of these proto-oncogenes and delineate the mechanism by which growth factor signaling autonomy has been established. In this model, proliferation of the malignant cells, "T4-2", is driven by an autocrine loop not present in non-malignant, "S1", cells. This loop is upregulated at the earliest stage of progression toward malignancy in this model (S2) and consists of two growth factors, Amphiregulin and TGFβ. We show that TACE/ADAM17 activity is required for the function of these growth factors in both normal and malignant cells and that inhibition of this protease results in downregulation EGFR pathway activity and phenocopies EGFR inhibition by reverting the malignant phenotype of T4-2 cells in a 3D culture assay. Although TACE has many substrates, we demonstrate definitively that the reverting effect of TACE inhibition is a direct consequence of the inhibition of growth factor ectodomain shedding.

#3

AACR: Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications
San Diego, CA, Sept 21-25

Targeting TACE-Dependent Growth Factor Shedding in Breast Cancer

Paraic A. Kenny and Mina J. Bissell
Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley CA 94720

The ability to proliferate independently of signals from other cell types is a fundamental characteristic of tumor cells. Tumors resulting from inappropriate activation of the EGFR are common in multiple tissues and are, for the most part, refractory to current targeted therapies. Using a 3D culture model of human breast cancer progression, we have delineated a protease-dependent autocrine loop which provides an oncogenic stimulus to this pathway in the absence of proto-oncogene mutation. Inhibition of this protease, TACE/ADAM17, reverts the malignant phenotype by preventing mobilization of two crucial growth factors, Amphiregulin and TGF□. We further demonstrate a strong correlation between TACE and TGFβ expression in human breast cancers that is predictive of poor prognosis. These data implicate TACE as a therapeutically tractable enzyme, the inhibition of which effectively blocks EGFR signaling by preventing mobilization of ligands for this receptor and suggest that co-ordinate inhibition of TACE may augment the activity of EGFR inhibitors in a clinical setting.

#4

American Society for Cell Biology Annual Meeting, San Francisco, CA, Dec 10-14, 2005

Targeting TACE-Dependent Growth Factor Shedding in Breast Cancer

Paraic A. Kenny and Mina J. Bissell
Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley CA 94720

The ability to proliferate independently of signals from other cell types is a fundamental characteristic of tumor cells. Tumors resulting from inappropriate activation of the EGFR are common in multiple tissues and are, for the most part, refractory to current targeted therapies. Using a 3D culture model of human breast cancer progression, we have delineated a protease-dependent autocrine loop which provides an oncogenic stimulus to this pathway in the absence of proto-oncogene mutation. Inhibition of this protease, TACE/ADAM17, reverts the malignant phenotype by preventing mobilization of two crucial growth factors, Amphiregulin and TGFβ. We show further that the efficacy of EGFR inhibitors is overcome by physiological levels of growth factors and that successful EGFR inhibition is dependent on reducing ligand bioavailability. Using existing patient outcome data, we demonstrate a strong correlation between TACE and TGF□ expression in human breast cancers that is predictive of poor prognosis. These data implicate TACE as a therapeutically tractable enzyme, the inhibition of which effectively blocks EGFR signaling by preventing mobilization of ligands for this receptor. Furthermore, the data provide mechanistic insight into the insensitivity of EGFR-overexpressing tumors to anilinoquinazoline inhibitors and suggest that co-ordinate inhibition of TACE might augment the activity of EGFR inhibitors in a clinical setting.

#5

AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics, Philadelphia, PA, Nov 14-18.

Targeting TACE-dependent Growth Factor Shedding in Breast Cancer

Paraic A. Kenny and Mina J. Bissell
Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley CA 94720

The ability to proliferate independently of signals from other cell types is a fundamental characteristic of the tumor cell. Acquisition of this phenotype is likely necessary in all cancers, but the preferred mechanism by which it is achieved is somewhat tissue specific. While common in other epithelial tumors, mutations in components downstream of the EGFR such as Ras and Raf are rare in breast tumors. Here, we analyze a model of human breast cancer progression in which this transition has occurred without mutation of these proto-oncogenes and delineate the mechanism by which growth factor signaling autonomy has been established. In this model, proliferation of the malignant cells, "T4-2", is driven by an autocrine loop not present in non-malignant, "S1", cells. This loop is upregulated at the earliest stage of progression toward malignancy in this model (S2) and consists of two growth factors, Amphiregulin and TGFβ. We show that TACE/ADAM17 activity is required for the function of these growth factors in both normal and malignant cells and that inhibition of this protease results in downregulation EGFR pathway activity and phenocopies Iressa-induced EGFR inhibition by reverting the malignant phenotype of T4-2 cells in a 3D culture assay. Although TACE has many substrates, we demonstrate definitively that the reverting effect of TACE inhibition is a direct consequence of the inhibition of growth factor ectodomain shedding. We further demonstrate a strong positive correlation between TACE and TGFβ expression in human breast cancers that is predictive of poor prognosis. These data implicate TACE as a therapeutically tractable enzyme, the inhibition of which effectively blocks EGFR signaling by preventing mobilization of ligands for this receptor and suggest that co-ordinate inhibition of TACE may augment the activity of EGFR inhibitors in a clinical setting.

Appendix 4

Invited Oral Presentations

#1

UCSF Breast Oncology Program Annual Retreat, Feb 2004

Inhibition of TACE-dependent growth factor shedding in a breast cancer model: an alternative to kinase inhibition?

Paraic A. Kenny and Mina J. Bissell

Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley CA 94720

The ability to proliferate independently of signals from other cell types is a fundamental characteristic of the tumor cell. Acquisition of this phenotype is likely necessary in all cancers, but the preferred mechanism by which it is achieved is somewhat tissue specific. While common in other epithelial tumors, mutations in components of the EGFR pathway such as Ras and Raf are rare in breast tumors. Here, we analyze a model of human breast cancer progression in which this transition has occurred and delineate the mechanism by which growth factor signaling autonomy has been established. In this model, proliferation of the malignant cells, "T4-2", is driven by an autocrine loop not present in non-malignant, "S1", cells. This loop is upregulated at the earliest stage of progression toward malignancy in this model (S2) and consists of two growth factors, Amphiregulin and TGFβ, and TACE, a member of the ADAM family of metalloproteinases. We show that TACE activity is required for the function of these growth factors in both normal and malignant cells and that inhibition of TACE results in downregulation EGFR pathway activity and phenocopies EGFR inhibition by reverting the malignant phenotype of T4-2 cells in a 3D culture assay. Although TACE has many substrates, we demonstrate definitively that the reverting effect of TACE inhibition is a direct consequence of the inhibition of growth factor ectodomain shedding.

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Coordinate upregulation of growth factors and TACE mediates escape from EGF dependence in the HMT3522 breast tumor progression series

Paraic A. Kenny and Mina J. Bissell

Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley CA 94720

The HMT-3522 cell series originated from a purified human mammary epithelial cell population and was passaged sequentially for ten years. Early passages, termed S1, are EGF-dependent, non-malignant and form phenotypically normal, growth-arrested, polarized structures which are similar in size and morphology to the acini of the human breast when cultured in a 3D reconstituted basement membrane. Serial passage in the absence of EGF resulted in the eventual outgrowth of a population (T4-2), that is malignant *in vivo* and forms disorganized continuously-growing apolar colonies in 3D lrBM culture. Here we report that two EGFR ligands, Amphiregulin and TGF-alpha are upregulated in T4-2 cells, but that their overexpression is insufficient to confer growth factor autonomy on non-malignant cells. We show that TACE/ADAM17 is upregulated in T4-2 cells, and that its proteolytic activity is functionally required for mobilization of endogenously produced growth factors. Analysis of cell lines of intermediate phenotype reveals that establishment of this autocrine loop preceded the transition to malignancy, but is critically required for the malignant phenotype. Experiments are currently underway to determine the consequences of shRNA-mediated suppression of these genes in malignant cells. These investigations were supported by a Susan G. Komen Foundation Postdoctoral Fellowship (#2000-223) to PAK and by grants from the OBER office of the DOE, the NCI, and an Innovator Award and training grants from the DOD to MJB.